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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/042,614	01/09/2002	Ya Fang Liu	YFLU-P03-001	6176
23628	7590	02/02/2006	EXAMINER	
WOLF GREENFIELD & SACKS, PC FEDERAL RESERVE PLAZA 600 ATLANTIC AVENUE BOSTON, MA 02210-2211			HANLEY, SUSAN MARIE	
			ART UNIT	PAPER NUMBER
			1651	

DATE MAILED: 02/02/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/042,614	Applicant(s) LIU, YA FANG	
	Examiner Susan Hanley	Art Unit 1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 19 October 2005.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 33,34 and 44-47 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 33,34 and 44-47 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date <u>10/19/05</u> . | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

The response and amendment filed 10/19/05 are acknowledged.

Claims 33, 34 and 44-47 are pending.

Response to Arguments

Applicant's arguments with respect to claims 33, 34 and 44-47 have been considered but are moot in view of the new ground(s) of rejection.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 33, 34 and 44-47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 33 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: The claim lacks a step that correlates the result of the *in vitro* inhibition assay of parts a) and b) with the determination of apoptosis in the neuronal tissue sample, parts c) to e). In order to assess the ability of the test compound to specifically inhibit JNK in a mammal having or susceptible to a neurological condition, the claim must have a step that makes a correlation between the results of the *in vitro* and *in vivo* results. Otherwise, one cannot make a positive or negative conclusion regarding the relationship of the *in vitro* JNK-inhibitory activity of the test compound as it relates to the *in vivo* JNK-inhibitory activity of apoptosis.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 33, 34, 44 and 47 are rejected under 35 U.S.C. 102(e) as being clearly anticipated by Davis et al. (US 6,943,000; “Davis”).

Davis discloses *in vitro* and *in vivo* methods for screening inhibitors of JNK3 for diseases involving excitotoxicity such as Alzheimer’s disease, Huntington Disease, Parkinson’s disease and ischemia (abstract). Candidate compounds can be tested in cell or tissue culture as well as animal models. Davis teaches, for example, that cells expressing JNK3 can be incubated with the test compound. Lysates can be prepared from treated and untreated cells and Western blotted. Antibodies for JNK3 can be used to assess the amount of JNK3 expression in the treated and control cells. Alternatively, test compounds can be administered to cell cultures with radiolabelled ATP. The amount of phosphorylation of a JNK3 substrate can be measured and compared to a control (col. 9, lines 55-65). The radiolabelled phosphorylation assay can also be accomplished by incubating an isolated JNK3 and a JNK substrate such as ATF2, Elk-1 (col. 26, lines 35-64) or c-Jun (col. 10, lines 29-31). This disclosure meets the limitations of instant claim 33, parts a and b, because Davis discloses incubating a test compound with a JNK and its substrate, wherein the degree of the phosphorylation of the JNK substrate is a measure of its ability to inhibit JNK. The disclosure of the use of c-Jun and a phosphate donor meets the limitations of instant claim 47. The disclosure of JNK3 meets the limitation of instant claim 34 regarding the isoforms type of JNK.

Test compounds predicted to inhibit JNK3 activity can be administered to animals as models for the various disease paradigms. The treated animal is then assayed for inhibition of the JNK activity by observation of the animal or by directly measuring JNK3 expression in neural tissue removed from the animal and comparing the result to a control (col. 10-11, bridging paragraph). Davis also discloses that neuronal apoptosis can also be assessed by TUNEL assay to evaluate whether a JNK3 modulator is affecting apoptosis (col. 25, lines 23-26). Davis demonstrated the utility of TUNEL to assess apoptosis associated with JNK3 modulates by administering kainic acid (KA) to two sets of mice. One set of mice was positive for hippocampal neurons expressing JNK3 while the other set was deficient. After KA administration, the mice were sacrificed and the hippocampal tissue was stained with crystal violet and subjected to TUNEL assay. The tissue from mice having normal JNK3 expression exhibited apoptosis while JNK3-negative mice had normal hippocampal regions (see Example 7, columns 23-25). This disclosure meets the limitations of instant claim 33, parts c-e because Davis teaches the administration of a compound to an animal, harvesting neuronal tissue, determining apoptosis in the sample and comparing the result to a control.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 33, 44 and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Park et al. (US 6,087,366; "Park") in view of Davis et al. (US 6,943,000; "Davis").

Park discloses a method of assessing the ability of a compound to specifically inhibit JNK kinase activity *in vitro* as well as a method to determining the ability of a compound to inhibit neuronal apoptosis in cell culture. Park teaches that flavopiridol or olomoucine were incubated with neuronally differentiated (pre-exposure to NGF) to assess their ability to block cell death upon withdrawal of NGF (col. 9, lines 12-25). The ability of flavopiridol or olomoucine to directly affect JNK activity *in vitro* was

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investigated by incubating c-JUN with and without the test compounds and measuring c-Jun kinase activity by determining the incorporation of ^{32}P from radiolabelled ATP into the c-Jun substrate (col. 8, lines 23-44). Park reports that flavopiridol or olomoucine inhibit neuronal cell death but not by directly inactivating JNK. This conclusion was based on the data that both compounds were found to be relatively poor inhibitors of JNK (col. 13, lines 10-23). Therefore, Park discloses that it was known in the art at the time the invention was made to assess the ability of test compounds to inhibit neuronal death by comparing the comparing the ability of a compound to inhibit neuronal apoptosis in PC12 cultures (see Figs. 1B and 2B) by testing test compounds in cell cultures and to determine if there is a correlation with direct inhibition of JNK by measuring the direct inhibition of JNK via a radiolabelled assay. Park teaches that flavopiridol or olomoucine can be used to treat neurological disorders such as Parkinson's, AML and Alzheimer's disease (col. 6, lines 43-46).

Park does not disclose that the test compound's ability to inhibit neuronal apoptosis was determined by administering the compound to an animal, harvesting neuronal tissue from said mammal, determining apoptosis in the tissue sample compared to a baseline sample to determine the compound's ability to specifically inhibit JNK in the mammal.

The disclosure taught by Davis is discussed *supra*.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to assess the ability of a compound to specifically inhibit the ability of a compound to inhibit JNK activity in a mammal susceptible to or having a neuronal condition by performing the in vitro assay with isolated JNK taught by Park and correlating it with the TUNEL methodology taught by Davis. The ordinary artisan would have been motivated to do so because the TUNEL method more reliably predicts the ability of the test compound to treat the disease in a target human population. Park teaches the employment of in vitro assays with isolated JNK as well as cell culture assays to determine the activity of the test compound. However, the ordinary artisan would have realized that a disease model which employs a live animal is a better predictor of the likely efficacy in the actual human target. It is well

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known in the art that clinical trials for testing drug leads progress from *in vitro* to *in vivo* to assess the efficacy and safety of a potential drug. The ordinary artisan would have had a reasonable expectation that the TUNEL method of assessing apoptosis taught by Davis would provide a reliable *in vivo* method for assessing the ability of a compound to specifically inhibit JNK because Davis correlated apoptosis measured by the TUNEL method to JNK inhibition.

Claims 33, 44, 46 and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Park et al. (US 6,087,366; "Park") in view of Davis et al. (US 6,943,000; "Davis"), as applied to claims 33, 44 and 47, in further view of Liu (1997; cited in the Office Action mailed on 6/14/05).

The combined disclosures by Park and Davis are discussed *supra*.

The combined disclosures do not teach using the dye Hoechst 33342 to assess apoptosis in the TUNEL method.

Liu et al. disclose that TUNEL and the staining of cells with Hoechst 33342 is a common alternative to determine neuron apoptosis (p. 5396, left column, see Histopathology section).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ the dye Hoechst 33342 for staining of cells in the TUNEL with to assess apoptosis in the modified method suggested by Davis and Park. The ordinary artisan would have been motivated to do so because the application of the dye Hoechst 33342 is at least an equivalent alternatives to the crystal violet dye employed by Davis for assessing neuronal apoptosis. The ordinary artisan would have had a reasonable expectation that the dye Hoechst 33342 could be used to assess apoptosis in the TUNEL method because Liu successfully demonstrated that the dye Hoechst 33342 adequately stains cells for TUNEL analysis of apoptosis in neurons.

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Claims 33, 34, 44, 46 and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis et al. (US 6,943,000; "Davis"), as applied to claims 33, 34, 44 and 47, in view of Liu (1997; cited in the Office Action mailed on 6/14/05).

The disclosures by Davis is discussed *supra*.

Davis does not teach using the dye Hoechst 33342 to assess apoptosis in the TUNEL method.

Liu et al. disclose that TUNEL and the staining of cells with Hoechst 33342 is a common alternative to determine neuron apoptosis (p. 5396, left column, see Histopathology section).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ the dye Hoechst 33342 for staining of cells in the TUNEL with to assess apoptosis in the modified method of Davis. The ordinary artisan would have been motivated to do so because the application of the dye Hoechst 33342 is at least an equivalent alternatives to the crystal violet dye employed by Davis for assessing neuronal apoptosis. The ordinary artisan would have had a reasonable expectation that the dye Hoechst 33342 could be used to assess apoptosis in the TUNEL method because Liu successfully demonstrated that the dye Hoechst 33342 adequately stains cells for TUNEL analysis of apoptosis in neurons.

Claims 33, 44, 45 and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Park et al. (US 6,087,366; "Park") in view of Davis et al. (US 6,943,000; "Davis"), as applied to claims 33, 44 and 47, in further view of Gnegy et al. (1976, "Gnegy").

The combined disclosures by Park and Davis are discussed *supra*.

The combined disclosures do not teach that ability of the test compound to specifically inhibit JNK can be correlated by measuring the *in vitro* phosphorylation of c-Jun by JNK in the presence of the test compound and correlating it with the amount of ³²P-phosphorylated c-Jun in a neuronal tissue sample taken from a live animal that had received [γ -³²P]ATP and the test compound and comparing all to a control.

Gnegy discloses that *in vitro* and *in vivo* experiment were used to verify that phosphodiesterase protein activator (PDEA) is not the phosphorylation substrate of cAMP-dependent protein kinase. Rats were injected intraventricularly with [γ - ^{32}P]ATP and sacrificed four hours later. The brains were homogenized and the PDEA fractions were separated on polyacrylamide gel electrophoresis. The isolated PDEA band did not contain ^{32}P . Gnegy also teaches that PDEA was not an *in vitro* substrate for cAMP-dependent protein kinase. Therefore, Gnegy concluded that PDEA is not a phosphate acceptor for the phosphorylation reaction that triggers its release from an enriched membrane preparation(p. 354, right column, first and second paragraphs).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to determine the effect of a test compound on the phosphorylation of c-Jun *in vitro* by correlating the *in vitro* results with an *in vivo* test wherein [γ - ^{32}P]ATP and a test compound are administered to a live animal and then measuring the ^{32}P -incorporation into c-Jun. The ordinary artisan would have been motivated to do so because the method of administration of [γ - ^{32}P]ATP to a live animal, as disclosed by Gnegy, is a direct and reliable method for determining *in vivo* phosphorylation. The ordinary artisan would have realized that the method of Gnegy has the advantage of being less expensive and less technically difficult than the combined method of Park and Davis because the method of Gnegy does not require transgenic animals having altered JNK3 expression or the use of more sophisticated equipment for TUNEL assay (as disclosed by the combined disclosures of Park and Davis). Thus, the ordinary artisan would have known that the effect of a test compound on the *in vivo* phosphorylation of JNK3 could be more easily and less expensively determined using [γ - ^{32}P]ATP administration to a live animal.

The ordinary artisan would have had a reasonable expectation that the effect of a test compound on the *in vivo* phosphorylation of JNK3 could be determined using [γ - ^{32}P]ATP administration to a live animal and that the *in vivo* result could be correlated with an *in vitro* test. Gnegy showed that [γ - ^{32}P]ATP can be administered to a live animal and that one can determine if a particular protein is phosphorylated by determining the radioactive content of the desired protein on polyacrylamide gel electrophoresis.

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Finally, Gnegy correlated the *in vivo* result with the *in vitro* experiment that demonstrated that PDEA was not phosphorylated by cAMP-dependent protein kinase.

Claims 33, 34, 44, 45 and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis et al. (US 6,943,000; "Davis"), as applied to claims 33, 34, 44 and 47, in view of Gnegy et al. (1976, "Gnegy").

The disclosures by Davis is discussed *supra*.

Davis does not teach that ability of the test compound to specifically inhibit JNK can be correlated by measuring the *in vitro* phosphorylation of c-Jun by JNK in the presence of the test compound and correlating it with the amount of ^{32}P -phosphorylated c-Jun in a neuronal tissue sample taken from a live animal that had received $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and the test compound and comparing all to a control.

Gnegy discloses that *in vitro* and *in vivo* experiment were used to verify that phosphodiesterase protein activator (PDEA) is not the phosphorylation substrate of cAMP-dependent protein kinase. Rats were injected intraventricularly with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and sacrificed four hours later. The brains were homogenized and fractions were separated on polyacrylamide gel electrophoresis. The isolated PDEA band did not contain ^{32}P . Gnegy also teaches that PDEA was not an *in vitro* substrate for cAMP-dependent protein kinase. Therefore, Gnegy concluded that PDEA is not a phosphate acceptor for the phosphorylation reaction that triggers its release from an enriched membrane preparation(p. 354, right column, first and second paragraphs).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to determine the effect of a test compound on the phosphorylation of c-Jun *in vitro* by correlating the *in vitro* results with an *in vivo* test wherein $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and a test compound are administered to a live animal and then measuring the ^{32}P -incorporation into c-Jun. The ordinary artisan would have been motivated to do so because the method of administration of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ to a live animal, as disclosed by

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Gnegy, is a direct and reliable method for determining *in vivo* phosphorylation. The ordinary artisan would have realized that the method of Gnegy has the advantage of being less expensive and less technically difficult than the method of Davis because the method of Gnegy does not require transgenic animals having altered JNK3 expression or the use of more sophisticated equipment for TUNEL assay. Thus, the ordinary artisan would have known that the effect of a test compound on the *in vivo* phosphorylation of JNK3 could be more easily and less expensively determined using [γ - 32 P]ATP administration to a live animal.

The ordinary artisan would have had a reasonable expectation that the effect of a test compound on the *in vivo* phosphorylation of JNK3 could be determined using [γ - 32 P]ATP administration to a live animal and that the *in vivo* result could be correlated with an *in vitro* test. Gnegy showed that [γ - 32 P]ATP can be administered to a live animal and that one can determine if a particular protein is phosphorylated by determining the radioactive content of the desired protein on polyacrylamide gel electrophoresis. Finally, Gnegy correlated the *in vivo* result with the *in vitro* experiment that demonstrated that PDEA was not phosphorylated by cAMP-dependent protein kinase.

No claim is allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The following prior art is relevant to the cited patents of Park et al. (US 6,087,366) and Davis et al. (US 6,943,000).

Yang et al. "Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the JNK3 gene" *Nature* (1997) 389: 865-870.

Park et al. "Ordering the cell death pathway" *J. Biol. Chem.* (1996) 271(36): 21898-21905.

Park et al. "Inhibitors of cyclin-dependent kinases promote survival of post-mitotic neuronally differentiated PC12 cells and sympathetic neurons" *J. Biol. Chem.* (1996) 271(14): 8161-8169.

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Conclusion

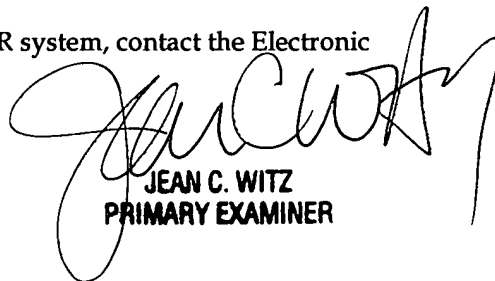
Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan Hanley whose telephone number is 571-272-2508. The examiner can normally be reached on M-F 9:00-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).


JEAN C. WITZ
PRIMARY EXAMINER